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☐ 1. 20030113355 . 11 Sep 02. 19 Jun 03. Nontoxic vernix compositions and method of producing. Hoath, Steven B., et al. 424/401; A61K007/00.

☑ 2. 20020187498 . 01 Mar 02. 12 Dec 02. Skin substitutes for irritancy testing. Comer, Allen, et al. 435/6; 435/371 C12Q001/68 C12N005/08.

☐ 3. 20020168768 . 01 Mar 02. 14 Nov 02. Skin substitutes with improved barrier function. Comer, Allen, et al. 435/371; 424/93.7 C12N005/08 A61K045/00.

☑ 4. 20020164793 . 01 Mar 02. 07 Nov 02. Skin substitutes and uses thereof. Conrad, Paul Barth, et al. 435/371; 424/93.7 C12N005/08 A61K045/00.

☐ 5. 6562358 . 08 May 01; 13 May 03. Nontoxic vernix compositions and method of producing. Hoath; Steven B., et al. 424/402; 424/401 424/444 424/445 424/59. A01N025/35.

☐ 6. 6514711 . 31 Aug 01; 04 Feb 03. Immortalized human keratinocyte cell line. Allen-Hoffmann; B. Lynn. 435/7.21; 435/347 435/371 435/373 435/402 435/408. G01N033/567 C12N005/08.

☐ 7. 6113932 . 25 Feb 99; 05 Sep 00. Nontoxic vernix compositions and method of producing. Hoath; Steven B., et al. 424/402; 424/401 424/443 424/444 424/445 424/59. A01N025/35.

☐ 8. 5989577 . 02 Mar 98; 23 Nov 99. Nontoxic vernix compositions and method of producing. Hoath; Steven B., et al. 424/402; 424/401 424/443 424/444 424/445 424/59. A01N025/34.

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FILE 'MEDLINE, CAPLUS, BIOSIS, SCISEARCH' ENTERED AT 19:16:00 ON 03 SEP 2003

L1 4846 S ARTIFICIAL(3A)SKIN OR SKIN(3A)(SUBSTITUTE OR EQUIVALENT)
L2 595 S SURFACE(3A)ELECTRI?(3A)CAPACIT?
L3 24 S L1 AND L2
L4 28340 S CERAMIDE
L5 0 S L3 AND L4
L6 73698 S KERATINOCYTE OR NIKS
L7 22 S L3 AND L6
L8 9 DUP REM L7 (13 DUPLICATES REMOVED)
L9 10 DUP REM L3 (14 DUPLICATES REMOVED)

=> d au ti so ab 1-9 l8

L8 ANSWER 1 OF 9 MEDLINE on STN DUPLICATE 1
AU Boyce Steven T; Supp Andrew P; Swope Viki B; Warden Glenn D
TI Vitamin C regulates **keratinocyte** viability, epidermal barrier, and basement membrane in vitro, and reduces wound contraction after grafting of cultured **skin substitutes**.
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (2002 Apr) 118 (4) 565-72. Journal code: 0426720. ISSN: 0022-202X.
AB Cultured **skin substitutes** have become useful as adjunctive treatments for excised, full-thickness burns, but no **skin substitutes** have the anatomy and physiology of native skin. Hypothetically, deficiencies of structure and function may result, in part, from nutritional deficiencies in culture media. To address this hypothesis, vitamin C was titrated at 0.0, 0.01, 0.1, and 1.0 mM in a cultured **skin substitute** model on filter inserts. Cultured **skin substitute** inserts were evaluated at 2 and 5 wk for viability by incorporation of 5-bromo-2'-deoxyuridine (BrdU) and by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) conversion. Subsequently, cultured **skin substitute** grafts consisting of cultured human **keratinocytes** and fibroblasts attached to collagen-glycosaminoglycan substrates were incubated for 5 wk in media containing 0.0 mM or 0.1 mM vitamin C, and then grafted to athymic mice. Cultured **skin substitutes** (n = 3 per group) were evaluated in vitro at 2 wk of incubation for collagen IV, collagen VII, and laminin 5, and through 5 wk for epidermal barrier by **surface electrical capacitance**. Cultured **skin substitutes** were grafted to full-thickness wounds in athymic mice (n = 8 per group), evaluated for **surface electrical capacitance** through 6 wk, and scored for percentage original wound area through 8 wk and for HLA-ABC-positive wounds at 8 wk after grafting. The data show that incubation of cultured **skin substitutes** in medium containing vitamin C results in greater viability (higher BrdU and MTT), more complete basement membrane development at 2 wk, and better epidermal barrier (lower **surface electrical capacitance**) at 5 wk in vitro. After grafting, cultured **skin substitutes** with vitamin C developed functional epidermal barrier earlier, had less wound contraction, and had more HLA-positive wounds at 8 wk than without vitamin C. These results suggest that incubation of cultured **skin substitutes** in medium containing vitamin C extends cellular viability, promotes formation of epidermal barrier in vitro, and promotes engraftment. Improved anatomy and physiology of cultured **skin substitutes** that result from nutritional factors in culture media may be expected to improve efficacy in treatment of full-thickness skin wounds.

L8 ANSWER 2 OF 9 MEDLINE on STN DUPLICATE 2
AU Supp A P; Wickett R R; Swope V B; Harriger M D; Hoath S B; Boyce S T
TI Incubation of cultured **skin substitutes** in reduced humidity promotes cornification in vitro and stable engraftment in athymic mice.
SO WOUND REPAIR AND REGENERATION, (1999 Jul-Aug) 7 (4) 226-37. Journal code: 9310939. ISSN: 1067-1927.
AB Cultured **skin substitutes** have been used successfully for adjunctive treatment of excised burns and chronic skin wounds. However, limitations inherent to all models of cultured skin include deficient barrier function in vitro, and delayed keratinization after grafting in comparison to native skin autografts. Experimental conditions for incubation of **skin substitutes** were tested to stimulate barrier development before grafting, and measure responses in function and stability after grafting. Cultured **skin substitutes** consisted of human **keratinocytes** and fibroblasts attached to collagen-glycosaminoglycan biopolymer substrates. Parallel cultured **skin substitutes** were incubated at the air-liquid interface in ambient (48-61%) or saturated (79-91%) relative humidity, and grafted to athymic mice on culture day 14. Additional cultured **skin substitutes** were incubated in the experimental conditions for a total of 28 days. Cadaveric human skin and acellular biopolymer substrates served as controls. Epidermal barrier was evaluated as the change in **surface** hydration by **surface electrical capacitance** with the NOVA Dermal Phase Meter. Cultured **skin substitutes** and cadaveric **skin** incubated in ambient humidity had lower baseline **surface electrical capacitance** and less change in **surface electrical capacitance** than parallel samples incubated in saturated humidity at all time points in vitro. Data from healing cultured **skin substitutes** at 2, 4, 8 and 12 weeks after grafting showed an earlier return to hydration levels comparable to native human skin, and more stable engraftment for **skin substitutes** from ambient humidity. The data indicate that cultured **skin substitutes** in ambient humidity have lower **surface electrical capacitance** and greater stability in vitro, and that they reform epidermal barrier more rapidly after grafting than cultured **skin substitutes** in saturated humidity. These results suggest that restoration of functional epidermis by cultured **skin substitutes** is stimulated by incubation in reduced humidity in vitro.

L8 ANSWER 3 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AU Boyce, S. T.; Swope, V.; Supp, A. P.; Warden, G. D.
TI Fibroblasts in cultured **skin substitutes** stimulate epidermal barrier and increase cellular viability.
SO Journal of Burn Care & Rehabilitation, (Jan.-Feb., 1999) Vol. 20, No. 1 PART 2, pp. S197.
Meeting Info.: 31st Annual Meeting of the American Burn Association Lake Buena Vista, Florida, USA March 24-27, 1999 American Burn Association . ISSN: 0273-8481.

L8 ANSWER 4 OF 9 MEDLINE on STN DUPLICATE 3
AU Boyce S T; Supp A P; Swope V B; Warden G D
TI Topical sulfamylon reduces engraftment of cultured **skin substitutes** on athymic mice.
SO JOURNAL OF BURN CARE AND REHABILITATION, (1999 Jan-Feb) 20 (1 Pt 1) 33-6. Journal code: 8110188. ISSN: 0273-8481.
AB Sulfamylon (mafenide acetate) remains extremely valuable for the control of the bacterial contamination of burn wounds, but it is cytotoxic to cultured **keratinocytes** used for wound closure. Because composite **skin substitutes** develop a partial epidermal barrier in vitro, they may hypothetically tolerate the use of topical

Sulfamylon. To test this hypothesis, cultured **skin substitutes** were prepared from cultured human fibroblasts; **keratinocytes** were attached to these collagen-based substrates, which were grafted to full-thickness wounds in athymic mice (n = 8 per group). Wounds were irrigated twice daily with 5% (wt/vol) Sulfamylon solution or with a formulation of noncytotoxic antimicrobials (0% Sulfamylon). On day 9 after grafting, the wounds were treated with dry dressings and assessed at 4 weeks for expression of human leukocyte antigens-A, B, C and at 2, 3, and 4 weeks for percentage of original wound area and **surface electrical capacitance** in picofarads (pF). Data were analyzed for statistical significance (P < .05) by Fisher's exact test, Student's t test, and repeated measures analysis of variance: [table: see text] The data demonstrate that irrigation of cultured **skin substitutes** with a solution of 5% Sulfamylon results in smaller wound area, fewer wounds that contain human cells, and greater **surface** hydration (higher **surface electrical capacitance**) than irrigation with noncytotoxic antimicrobial agents. These results support the conclusion that cultured **skin substitutes** of this type do not tolerate the chemical toxicity of Sulfamylon as well as skin autografts. Further improvements in the properties of the epidermal barrier of cultured **skin substitutes** may facilitate the use of Sulfamylon or other potent antimicrobial agents for the management of microbial contamination during engraftment of transplanted skin cells.

L8 ANSWER 5 OF 9 MEDLINE on STN DUPLICATE 4
AU Boyce S T
TI **Skin substitutes** from cultured cells and collagen-GAG polymers.
SO MEDICAL AND BIOLOGICAL ENGINEERING AND COMPUTING, (1998 Nov) 36 (6) 791-800. Ref: 101
Journal code: 7704869. ISSN: 0140-0118.
AB Engineering **skin substitutes** provides a potential source of advanced therapies for the treatment of acute and chronic wounds. Cultured **skin substitutes** (CSS) consisting of human **keratinocytes** and fibroblasts attached to collagen-glycosaminoglycan substrates have been designed and tested in preclinical and clinical studies. Cell culture techniques follow general principles of primary culture and cryopreservation in liquid nitrogen for long-term storage. Biopolymer substrates are fabricated from xenogeneic (bovine) collagen and glycosaminoglycan that are lyophilised for storage until use. At maturity in air-exposed culture, CSS develop an epidermal barrier that is not statistically different from native human skin, as measured by **surface electrical capacitance**.
Preclinical studies in athymic mice show rapid healing, expression of cytokines and regulation of pigmentation. Clinical studies in burn patients demonstrate a qualitative outcome with autologous skin that is not different from 1:4 meshed, split-thickness autograft skin, and with a quantitative advantage over autograft skin in the ratio of healed skin to biopsy areas. Chronic wounds resulting from diabetes or venous stasis have been closed successfully with allogeneic CSS prepared from cryopreserved skin cells. These results define the therapeutic benefits of cultured **skin substitutes** prepared with **skin** cells from the patient or from cadaver donors. Future directions include genetic modification of transplanted cells to improve wound healing transiently or to deliver gene products systemically.

L8 ANSWER 6 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AU Boyce, S. T. (1); Swope, V. B.; Supp, A. P.; Warden, G. D.
TI Vitamin C promotes epidermal barrier and DNA synthesis in **keratinocytes** of cultured **skin substitutes**.
SO Molecular Biology of the Cell, (Nov., 1997) Vol. 8, No. SUPPL., pp. 339A.
Meeting Info.: 37th Annual Meeting of the American Society for Cell

Biology Washington, D.C., USA December 13-17, 1997 American Society for Cell Biology
. ISSN: 1059-1524.

L8 ANSWER 7 OF 9 MEDLINE on STN DUPLICATE 5
AU Harriger M D; Supp A P; Swope V B; Boyce S T
TI Reduced engraftment and wound closure of cryopreserved cultured **skin substitutes** grafted to athymic mice.
SO CRYOBIOLOGY, (1997 Sep) 35 (2) 132-42.
Journal code: 0006252. ISSN: 0011-2240.
AB Cryopreservation of cultured **skin substitutes** is a requirement for establishment of banks of alternative materials for treatment of acute and chronic skin wounds. To determine whether cryopreservation of **skin substitutes** that contain cultured cells reduces their efficacy for wound closure, cell-biopolymer grafts were frozen, recovered into culture, and grafted to wounds on athymic mice. Grafts consisted of cultured human **keratinocytes** and fibroblasts attached to collagen-glycosaminoglycan substrates that were frozen in cell culture medium with 20% serum and 10% DMSO at a controlled rate and stored overnight in liquid nitrogen. After recovery into culture for 24 h, frozen or unfrozen (control) **skin substitutes** were grafted to full-thickness wounds on athymic mice. Wound area and **surface electrical capacitance** were measured at 2, 3, and 4 weeks after grafting at which time animals were sacrificed. Wounds were scored for presence of human cells by direct immunofluorescence staining with a monoclonal antibody to HLA-ABC. The data demonstrate that cell-biopolymer grafts are less efficacious after controlled-rate cryopreservation using 10% DMSO as a cryoprotectant. Frozen grafts at 4 weeks after surgery have significantly smaller wound areas, higher capacitance (wetter surface), and fewer healed wounds that contain human cells. The results suggest that these conditions for cryopreservation of cultured grafts reduce graft viability. Improved conditions for cryopreservation are required to maintain viability and efficacy of cultured **skin substitutes** after frozen storage.

L8 ANSWER 8 OF 9 MEDLINE on STN DUPLICATE 6
AU Boyce S T; Supp A P; Harriger M D; Pickens W L; Wickett R R; Hoath S B
TI **Surface electrical capacitance** as a noninvasive index of epidermal barrier in cultured **skin substitutes** in athymic mice.
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY, (1996 Jul) 107 (1) 82-7.
Journal code: 0426720. ISSN: 0022-202X.
AB Restoration of an epidermal barrier is a definitive requirement for wound closure. To determine formation of an epidermal barrier as a function of hydration of the stratum corneum, we measured **surface electrical capacitance** (SEC) of the epidermis in cultured **skin substitutes** (CSS) in vitro and after grafting to athymic mice. CSS were prepared from human **keratinocytes** and fibroblasts attached to collagen-glycosaminoglycan substrates. On culture days 3, 7, 14, 17, and 21, SEC was measured in situ. CSS (n = 18; mean +/- SEM) showed a time-dependent decrease of SEC (picoFarads, "pF") from 4721 +/- 28 pF on day 3 to 394 +/- 117 pF on day 14, and subsequent increase to 1677 +/- 325 pF on day 21. After 14-d incubation, parallel CSS samples (n = 5) or murine autografts (n = 5) were grafted orthotopically to athymic mice. After grafting, CSS showed decreases in SEC from 910 +/- 315 pF at 2 wk to 40 +/- 10 pF at 4 wk with no significant decreases thereafter. Control values for murine autograft were 870 +/- 245 pF at 2 wk, and 87 +/- 30 pF at 4 wk. SEC values for native murine skin (n = 10) were 91 +/- 18 pF, and for native human skin (n = 10) were 32 +/- 5 pF. The data demonstrate that SEC decreases with time in culture and that healed or intact skin has approximately 10- to 100-fold lower SEC than CSS in vitro. This

noninvasive technique provides a quantitative index of epidermal barrier in CSS in vitro and demonstrates the development of functional epidermal barrier during healing of wounds treated with cultured **skin substitutes**.

L8 ANSWER 9 OF 9 BIOSIS COPYRIGHT 2003 BIOLOGICAL ABSTRACTS INC. on STN
AU Goretsky, Michael J.; Supp, Andrew P.; Greenhalgh, David G.; Warden, Glenn D.; Boyce, Steven T. (1)
TI **Surface electrical capacitance** as an index of epidermal barrier properties of composite **skin substitutes** and **skin** autografts.
SO Wound Repair and Regeneration, (1995) Vol. 3, No. 4, pp. 419-425. ISSN: 1067-1927.
AB Restoration of the epidermal barrier is a requirement for burn wound closure, A rapid, reliable, and noninvasive measure of the rate of restoration of the epidermal barrier is not readily available. To monitor the reformation of the epidermal barrier, we measured **surface electrical capacitance** on cultured **skin substitutes** (human **keratinocytes** and fibroblasts attached to collagen-glycosaminoglycan substrates) and split-thickness skin autografts grafted to patients. Data were collected from four patients with burns and one pediatric patient with a congenital hairy nevus comprising gt 60% total body surface area. Capacitance measurements were performed at days 7, 10, 12, 14, and 28 by direct contact of the capacitance probe for 10 seconds to the cultured **skin substitutes** or split-thickness autograft. On postoperative days 7, 10, 12,14, 21, and 28, the **surface electrical capacitance** of cultured **skin substitutes** after 10 seconds of sampling was 2468 +- 268, 1443 +- 439, 129 +- 43, 200 +- 44, 88 +- 20, and 74 +- 19 picofarads (mean +- standard error of the mean), respectively. **Surface electrical capacitance** for split-thickness autograft on the same days was 1699 +- 371, 1914 +- 433, 125 +- 16, 175 +- 63,110 +- 26, 271 +- 77 picofarads, respectively. **Surface electrical capacitance** in all of the grafts decreased with time, Cultured **skin substitutes** had approximately the same 10-second capacitance values as split-thickness autograft during 3 weeks of healing and approached values for uninjured skin (32 +- 5 picofarads) by 12 days. Measurement of **surface electrical capacitance** is a direct, inexpensive, and convenient index for noninvasive monitoring of epidermal barrier formation.

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